NASA TECHNICAL TRANSLATION NASA TT F-14,355

THE ABIOGENETIC FORMATION OF PORPHIN, CHLORIN, AND BACTERIOCHLORIN

A. A. Krasnovskiy, A. V. Umrikhina

Translation of "Abiogennoye obrazovaniye porfina, khlorina i bakteriokhlorina," Doklady Akademii Nauk SSR, Vol. 202, No. 1, 1972, pp. 221-224.

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
WASHINGTON, D. C. 20546 JULY 1972

THE ABIOGENETIC FORMATION OF PORPHIN, CHLORIN, AND BACTERIOCHLORIN

A. A. Krasnovskiy, A. V. Umrikhina

ABSTRACT: The use of model systems simulating prebiological conditions on earth to study the problem of the abiogenetic formation of porphyrins during the chemical evolution process is discussed.

The problem of the abiogenetic formation of porphyrins during the chemical /221* evolution process is studied by creating model systems simulating prebiological conditions on earth. A mixture of pyrrole and formaldehydre [1-5] is among such systems.

Methods. The reaction was made to take place in thick-walled glass ampules. The mixtures usually used were those of formaldehyde (2%) and pyrrole (0.5%) in methanol because they provided optimum reaction conditions. The ampules were filled, sealed, and heated in a boiling water bath. The appearance of red fluorescence of the porphyrins was traced as the reaction developed. Reaction products were purified and fractionated when the synthesis was completed, and the luminescent spectra of the reaction mixtures had been measured.

Influence of oxygen. We already have pointed out the accelerating effect of the oxygen in the air on porphin synthesis [2]. The ampules to which air was admitted began to fluoresce visibly after 15 to 20 minutes of heating, but several hours elapsed before the process occurred in those ampules to which no air was admitted. Two maxima (Figure 1a) in the fluorescent spectrum occur in all experiments in which air was present, at 685 and 633 mμ. There is a "shoulder" in the 620 mμ region (or a maximum if fluorescence at 633 mμ is lower) and an ill-defined "shoulder" in the 750 mμ region. The main maxima in a vacuum occur at 704 and 648 mμ. Thus, although the overall appearance of the spectrum in a vacuum and when air is present is the same, the positions of the fluorescence maxima in a vacuum are displaced 15 to 20 mμ to the longwave side. The shift in the spectrum can be explained by the formation of isomeric forms of porphyrins [6].

---

* Numbers in the margin indicate pagination in the foreign text.

An increase in the amount of water from 5 to 50% reduces the time from the beginning of heating to the appearance of fluorescence from 15 to 30 minutes to 1 to 2 minutes, but the final product yield decreases. The maximum at 633, or 648 mμ, respectively, is dominant in the fluorescent spectrum in a vacuum, as well as in the presence of air, at the beginning of the reaction in the presence of water.

Effect of impurities. The following have an accelerating effect on the synthesis of porphyrins: silicon dioxide, zinc oxide, oxides of iron, titanium dioxide, potassium chloride, polyvinyl pyrrolidone, and other compounds. The most active were zinc oxide (Figure 1b) and titanium dioxide. Ampule heating time to the appearance of red fluorescence in a vacuum was decreased from 5 to 7 hours to 15 to 30 minutes when catalyzers were present, and product yield increased as compared to the control experiment without catalyzer.

Electron donors. Ascorbic acid inhibited the synthesis of porphyin, whereas tryptophan actively catalyzed the reaction, and the tryptophan reaction can be observed even when the reacting mixtures have been cooled to 3 to 5°. Under these conditions, red fluorescence of porphyrins appeared within a fed days, but took only 15 to 30 minutes when heated. The yield of porphyrin compounds increased when the tryptophan was present. In air, the main maximum of fluorescence was found at 685 mμ (Figure 1c), with the fluorescence maxima at 704 and 643 mμ /222 in a vacuum. However, heating the solutions in the presence of an excess of tryptophan (10 mg per 2 ml of solution) resulted in the formation of products with blue fluorescence, possibly dipyrryl methenes. Use of tryptophan instead of pyrryl for the reaction does not lead to the formation of porphyrins.

Electron acceptors. The use of 1 mg of para-benzoquinone per 2 ml of solution accelerated the reaction of synthesis of porphyrins and increased product yield. In this case (in a vacuum, as well as when air was present) practically no compounds with maximum fluorescence at 633 mμ formed, nor was there a shift of the spectrum to the longwave side under vacuum conditions (Figure 1d), indicating the formation of the same isomeric form of porphyrins in a vacuum as in air.

Fractionation. Porphin, chlorin, and bacteriochlorin can be formed during the synthesis. Our data, as well as the data in the literature [7], put the

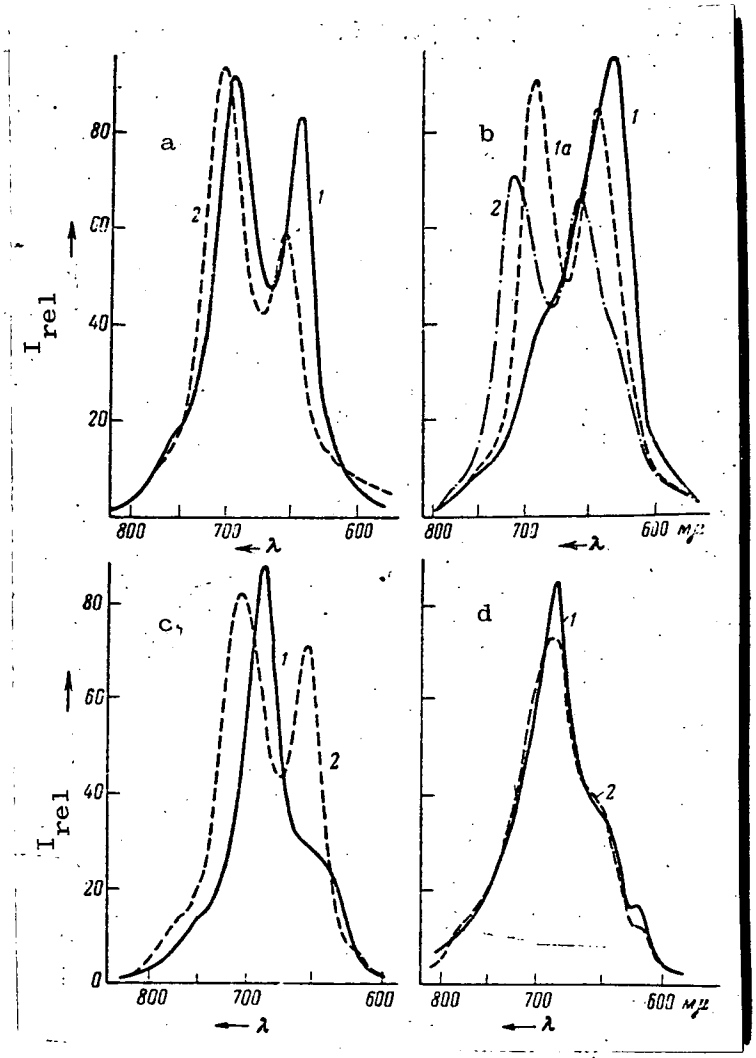


Figure 1. Fluorescent spectra observed (1- in air; 2- in a vacuum) upon heating solutions of pyrrole (0.5%) and formaldehyde (2%) in methanol (2 ml) under different conditions: a- control experiment; with additives as follows: b- 1 mg zinc oxide; c- 1 mg tryptophan; d- 1 mg quinone.

main maxima for absorption of porphin in nonpolar solvents at (1)617, (2)570, (3)520, and (4)490 mμ, with the distribution of intensities, in terms of the maxima, 4, 2, 3, 1 (a phyllotype of spectrum). Observed in the luminescent spectrum are two main bands at 690 and 620 mμ, and the fluorescence maximum is at 640 mμ. The absorption spectrum for the trans-form of tetraphenyl bacteriochlorin has maxima at 743 and 524 mμ, the cis-type at 600 and 650 mμ [8-10].

Thus, the fluorescence maximum we observed in our experiments at 685 and 520 mμ in air (and at 704 and 622 mμ in a vacuum) can be put down to the isomeric forms of porphin, and the maximum at 633 mμ (648 in a vacuum) to chlorin. The maximum at 764 mμ in concentrated solutions of pigments apparently can be attributed to the presence of bacteriochlorin.

Preliminary fractionation of the reaction products involved the extraction /223 of porphyrins at different stages of reducibility by solutions of hydrochloric acid. The conversion is primarily porphin [7] in 5% HCl, and chlorin in 10 and 20% solutions. The hydrochloric acid number for bacteriochlorin is unknown. Solutions of pigments in 5, 10, and 20% HCl were neutralized by sodium acetate. The pigments were transferred to a fresh batch of benzene, and the fluorescent spectra of fractions I, II, and III, as obtained, were measured. Fraction I, obtained from 5% HCl, contained primarily porphin (fluorescence maxima 685 and 620 mμ), but small maxima also were observed at 633 and 764 mμ. Fractions II and III (from 10 and 20% HCl) contained primarily chlorin, and the main maximum was at 633 mμ. Porphin was present in small amounts in these fractions, and whereas the original solution contained a product with a fluorescence maximum at 764 mμ, fractions II and III contained relatively more of it than did fraction I.

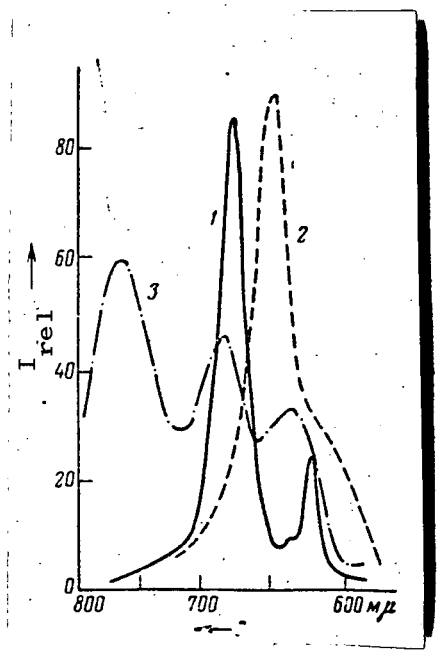


Figure 2. Fluorescent spectra of solutions of porphyrins in benzene after aluminum oxide chromatography: 1- porphin; 2- chlorin; 3- mixture of pigments with bacteriochlorin predominant.

Final separation of porphyrins was by Al_2O_3 column chromatography from a benzene solution. The eluate in benzene contained only porphin (Figure 2) when fraction I was chromatographed. The first batches of eluate obtained during the chromatography of fractions II and III contained primarily chlorin (Figure 2), with subsequent batches containing a mixture of porphin and chlorin, then porphin. The fluorescing zones of the adsorbent not eluted by the benzene were withdrawn from the column in separate batches, the pigments were removed from the aluminum oxide by pyridine, and the fluorescent spectra of the solutions obtained were measured. It was found that the main maximum of the fluorescence in the fluorescent spectrum of the upper and lower zones is at 764 mμ (Figure 2).

There still has been no success in completely refining the product with maximum fluorescence at 764 mμ (bacteriochlorin, supposedly). However, there is no 764 mμ maximum in the porphin or chlorin fluorescent spectra (Figure 2, curves 1 and 2) so it must belong to a third compound, the most likely one being bacteriochlorin. If the eluate with aluminum oxide in pyradine remains in air for a few days the intensity of fluorescence at the bacteriochlorin maximum of 764 mμ diminishes and the intensity of the fluorescence at 685 and 640 mμ increases, and this can be explained by the oxidation of the bacteriochlorin to prophin by the intermediate formation of chlorin. Known data [8-10] are informative with respect to the ease of oxidizability of bacteriochlorin pigments and the formation of chlorin and porphyrin compounds.

There is a maximum at 530 mμ (Figure 3) in the spectrum for the solution that is a mixture of porphyrins in benzene, which, by analogy with tetraphenyl bacteriochlorin probably is the second maximum of bacteriochlorin absorption. There is no maximum at 530 mμ in the spectrum for porphin and chlorin solutions.

Photoreduction of porphin. Observed in the course of the photoreduction of porphin by ascorbic acid in dry pyridine is the formation of products with an absorption maximum at 635 mμ and a fluorescence maximum at 643 mμ (Figure 4), which correspond to chlorin. The chlorin thus formed is stable in a vacuum, as well as when air is present.

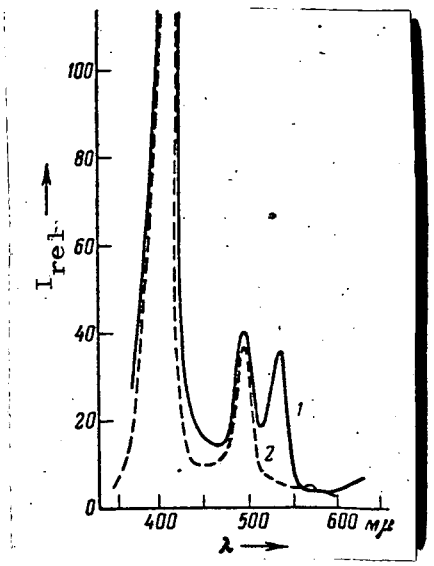


Figure 3. Spectra of excitation of luminescence of solutions of porphyrins in benzene. 1- mixture of pigments; 2- porphin.

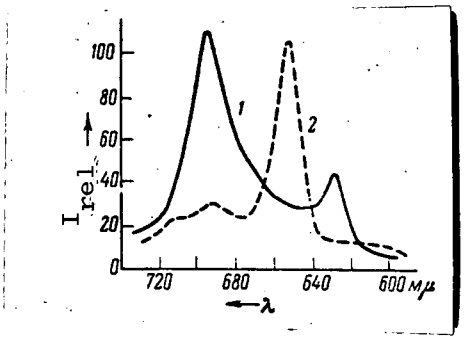
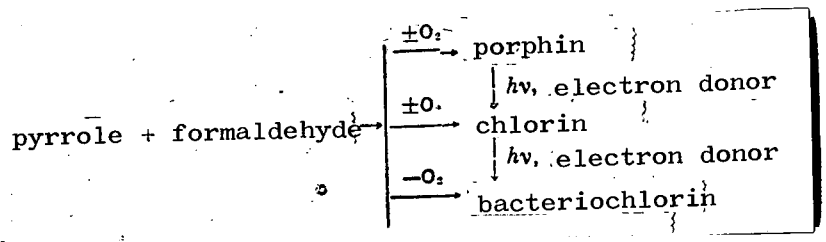


Figure 4. Spectra of fluorescence observed during the photoreduction of porphin by ascorbic acid (5 mg) in pyridine (4 ml) in a vacuum.
1- before illumination;
2- after 15 minutes of illumination.

The photoreduction of porphin by ascorbic acid in a vacuum in an acid (a soution of HCl in water, or in ethanol) proceeded by a sequential formation of forms with absorption maxima at 440, 500, and 625 mμ. The product with absorption at 625 mμ and fluorescence at 630 mμ corresponded, in its spectral and photochemical properties, to the acid form of chlorin [11].

The results of the work can be summarized by the following diagram



The formation of reduced forms of porphyrins can result from their synthesis from pyrrole predecessors, or from secondary photochemical, or dark, reduction by different electron donors. The data lead to the conclusion that the possibility of the formation of products with a different stage of reducibility, porphyrins, chlorins, and bacteriochlorins, should be taken into consideration during the search for abiogenetically formed porphyrins on other planets, or in the depths of the earth's crust.

A. N. Bakh Institute of Biochemistry
Academy of Sciences of the USSR, Moscow

Submitted
16 August 1971

REFERENCES

1. Rothemund, P., J. Am. Chem. Soc., 58, 1936, p. 625.

2. Krasnovskiy, A. A., Umrikhina, A. V., DAN, 155, 1964, p. 691.

3. Hodgson, G., Baker, B. L., Nature, 216, 1967, p. 29.

4. Szutka, A., Hazel, J. F., McNabb, W. M., Radiation Res., 10, 1959, p. 597.

5. Shutka, A., V sb. Proiskhozhdeniye predbiologicheskikh sistem [In collection: The Origin of Prebiological Systems], Moscow, 1966, p. 245.

6. Aronoff, S., Calvin, M., J. Organic Chem., 8, 1943, p. 205.

7. Rimington, C., Mason, S. F., Kennard, O., Spectrochim. acta, 12, 1958, p. 65.

8. Sidorov, A. N., Biofizika, 10, 1965, p. 226.

9. Seely, G. R., Talmadge, K., Photochem. Photobiol., 3, 1964, p. 195.

10. Krasnovskiy, A. A., Drozdova, N. N., Bokuchava, Ye. M., DAN, 190, 1970, p. 464.

11. Umrikhina, A. V., Yusupova, N. A., Krasnovskiy, A. A., DAN, 175, 1967, p. 1400.

Translated for the National Aeronautics and Space Administration under contract No. NASw-2038 by Translation Consultants, Ltd., 944 South Wakefield Street, Arlington, Virginia 22204.